

### 3. SUMMARY OF THE INVENTION

The present invention provides a polynucleotide and a polypeptide encoded thereby which has been identified as a phytase enzyme having phytase activity. In accordance with one aspect of the present invention, there is provided a novel recombinant enzyme, as well as active fragments, analogs and derivatives thereof.

More particularly, this invention relates to the use of recombinant phytase molecules of bacterial origin that are serviceable for improving the nutritional value of phytate-containing foodstuffs. Previous publications have disclosed the use of fungal phytases, but the use of bacterial phytases for this purpose is novel.

More particularly still, this invention relates to the use of newly identified recombinant phytase molecules of *E.coli* origin that are serviceable for improving the nutritional value of phytate-containing foodstuffs.

This use is comprised of employing the newly identified molecules to hydrolyze phytate in foodstuffs. Hydrolysis may occur before ingestion or after ingestion or both before and after ingestion of the phytate. This application is particularly relevant, but not limited, to non-ruminant organisms and includes the expression of the disclosed novel phytase molecules in transformed hosts, the contacting of the disclosed novel phytase molecules with phytate in foodstuffs and other materials, and the treatment of animal digestive systems with the disclosed novel phytase molecules.

Additionally, hydrolysis may occur independently of consumption, e.g. in an in vitro application, such as in a reaction vessel. Thus, the treatment of phytate-containing materials includes the treatment of a wide range of materials, including ones that are not intended to be foodstuffs, e.g. the treatment of excrementary (or fecal) material.

is administered the enzyme in the form of seeds from one or more plant species, preferably transgenic plant species, containing enhanced amounts of the enzyme. Additional details regarding this approach are in the public literature and/or are known to the skilled artisan. In a particular non-limiting exemplification, such publicly available literature includes **USPN 5,543,576** (Van Ooijen et al) and **USPN 5,714,474** (Van Ooijen et al), although these reference do not teach the inventive molecules of the instant application and instead teach the use of fungal phytases.

In a particular non-limiting aspect, the instant phytase molecules are serviceable for generating recombinant digestive system life forms (or microbes or flora) and for the administration of said recombinant digestive system life forms to animals. Administration may be optionally performed alone or in combination with other enzymes &/or with other life forms that can provide enzymatic activity in a digestive system, where said other enzymes and said life forms may be may recombinant or otherwise. For example, administration may be performed in combination with xylanolytic bacteria.

**6.3.3 - Steeping of cereals:** In a non-limiting aspect, the present invention provides a method for steeping corn or sorghum kernels in warm water containing sulfur dioxide in the presence of an enzyme preparation comprising one or more phytin-degrading enzymes, preferably in such an amount that the phytin present in the corn or sorghum is substantially degraded. The enzyme preparation may comprise phytase and/or acid phosphatase and optionally other plant material degrading enzymes. The steeping time may be 12 to 18 hours. The steeping may be interrupted by an intermediate milling step, reducing the steeping time. In a preferred embodiment, corn or sorghum kernels are steeped in warm water containing sulfur dioxide in the presence of an enzyme preparation including one or more phytin-degrading enzymes, such as phytase and acid phosphatases, to eliminate or greatly reduce phytic acid and the salts of phytic acid. Additional details regarding this approach are in the public literature and/or are known to the skilled artisan. In a particular non-limiting exemplification, such publicly available literature includes

cellulosomal domains should enable better use of cellulosic biomass and may offer a wide range of novel applications in research, medicine and industry.

In another non-limiting exemplification, the instant phytase molecules are serviceable - either alone or in combination treatments - in areas of biopulping and biobleaching where a reduction in the use of environmentally harmful chemicals traditionally used in the pulp and paper industry is desired. Waste water treatment represents another vast application area where biological enzymes have been shown to be effective not only in colour removal but also in the bioconversion of potentially noxious substances into useful bioproducts.

In another non-limiting exemplification, the instant phytase molecules are serviceable for generating life forms that can provide at least one enzymatic activity - either alone or in combination treatments - in the treatment of digestive systems of organisms. Particularly relevant organisms to be treated include non-ruminant organisms. Specifically, it is appreciated that this approach may be performed alone or in combination with other biological molecules (for example, xylanases) to generate a recombinant host that expresses a plurality of biological molecules. It is also appreciated that the administration of the instant phytase molecules &/or recombinant hosts expressing the instant phytase molecules may be performed either alone or in combination with other biological molecules, &/or life forms that can provide enzymatic activities in a digestive system - where said other enzymes and said life forms may be may recombinant or otherwise. For example, administration may be performed in combination with xylanolytic bacteria.

For example, in addition to phytate, many organisms are also unable to adequately digest hemicelluloses. Hemicelluloses or xylans are major components (35%) of plant materials. For ruminant animals, about 50% of the dietary xylans are degraded, but only small amounts of xylans are degraded in the lower gut of nonruminant animals and humans. In the rumen, the major xylanolytic species are *Butyrivibrio fibrisolvens* and *Bacteroides rumenicola*. In the human colon, *Bacteroides ovatus* and *Bacteroides fragilis*